THE ANABOLISM OF ASPARTIC ACID AND RELATED COMPOUNDS

BY

ROGER ERDMAN KOEPPE

A.B., Hope College, 1944

M.S., University of Illinois, 1947

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY IN THE GRADUATE COLLEGE OF THE UNIVERSITY OF ILLINOIS, 1950

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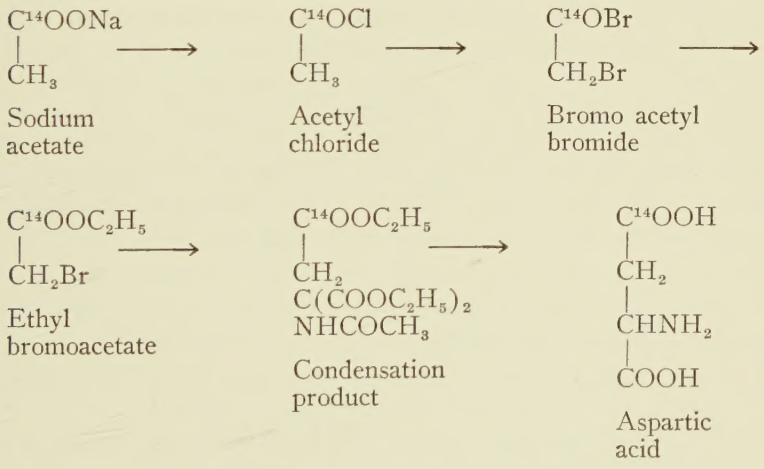
I. FATE IN THE ANIMAL ORGANISM OF ASPARTIC ACID LABELED WITH CARBON 14

Introduction

The metabolic origin of β-alanine in the intact animal is not known. This substance is present as a component of two muscle extractives, carnosine and anserine. It has been established that at least some lower organisms are capable of decarboxylating aspartic acid with the formation of β-alanine.[1,2,3,4] The purpose of the present study was to investigate the possible relationship of these two amino acids in the animal organism. Therefore, aspartic acid containing carbon 14 in the position beta to the amino group was synthesized and administered to a rabbit, following which anserine and several other tissue components were isolated and examined for radioactivity.

Experimental

The reactions involved in the synthesis of aspartic acid are indicated below.



The sodium acetate was converted to acetyl chloride by gently refluxing with phthalyl chloride. This product was then brominated with liquid bromine and treated with absolute ethanol to yield ethyl bromoacetate. The latter was condensed with ethyl acetamidomalonate in the presence of sodium ethoxide. Subsequent hydrolysis

of the condensation product gave a 44 per cent yield of labeled aspartic acid.

The tissue components were isolated as follows. Anserine was obtained as the copper salt from a water extract of the leg and back muscles.[5,6] Glycogen was precipitated from a trichloroacetic acid extract of the liver. Aspartic and glutamic acids were isolated from the liver and muscle proteins. The expired carbon dioxide was collected in sodium hydroxide traps and precipitated as barium carbonate. All the radioactivity measurements were made on carbon dioxide in an ionization chamber.

Following the injection of 200 mg. (175 microcuries) of the carbon 14-labeled aspartic acid into an adult rabbit, about 78 per cent of the isotope was recovered in the expired air. Slightly less than 2 per cent was found in the combined urine and feces.

The isotopic concentration of the liver glycogen was found to be two to four times that of the liver aspartic and glutamic acids. The latter, in turn, had ten to twenty times the specific activities of the muscle aspartic and glutamic acids.

The anserine was devoid of detectable activity. Thus, no evidence was obtained that the rabbit utilized the administered aspartic acid as a precursor of the β-alanine portion of this substance. However, the findings do not necessarily preclude the possibility that such a reaction may occur in the animal organism.

Summary

Aspartic acid labeled with carbon 14 has been synthesized and administered to a rabbit. About 80 per cent of the isotope was recovered from the excreta. The most active tissue component isolated was glycogen. No carbon 14 was detected in the anserine.

BIBLIOGRAPHY

1. Ackermann, D., Z. Biol., **56**, 87 (1911).
2. Virtanen, A. I., and Laine, T., Enzymologia, **3**, 266 (1937); Suomen Kemistilehti, **10B**, 2 (1937); Chem. abstr., **31**, 2631_2 (1937).
3. Shive, W., and Macow, J., J. Biol. Chem., **162**, 451 (1946). Ravel, J. M., and Shive, W., J. Biol. Chem., **166**, 407 (1946).
4. Billen, D., and Lichstein, H. C., J. Bact., **57**, 267 (1949); **58**, 215 (1949).
5. Ackermann, D., Timpe, O., and Poller, K., Z. physiol. Chem., **183**, 1 (1929).
6. Wolff, W. A., and Wilson, D. W., J. Biol. Chem., **95**, 495 (1932); **109**, 565 (1935).

II. THE L-THREONINE REQUIREMENT OF THE RAT FOR MAXIMUM GROWTH

Introduction

The first tentative report on the L-threonine requirement of the rat placed the minimum level at 0.6 per cent of the diet.[1,2] Since then, work in this laboratory has shown the requirement to be nearer 0.5 per cent.[3] These experiments were conducted with high fat and relatively low carbohydrate diets, in which the vitamin supplements were tiki-tiki and milk concentrate. More recent investigations have revealed the fact that more satisfactory growth data are obtained when the rations are low in fat. Furthermore, it is now feasible to employ crystalline vitamins instead of the crude preparations formerly used. In view of these facts, it seemed desirable to redetermine the L-threonine requirement of the rat.

Experimental

The subjects were male weanling rats from our stock colony. All tests were continued for twenty-eight days. The nitrogen portion of the diets was furnished as a mixture of analytically pure amino acids identical with Mixture XXIII [4], except for the omission of threonine. The latter was added separately as desired. The animals were allowed to consume food and water *ad libitum*. The weight gains and food consumption were measured at four-day intervals.

The growth rate of the animals given a diet containing 0.5 per cent of L-threonine was found to be significantly more rapid than the growth rate of litter mates maintained on a ration containing only 0.4 per cent of L-threonine. However, if the L-threonine level was raised to 0.6 per cent, the growth rate was not statistically greater than that manifested on the diet in which the level was 0.5 per cent. Hence, we may conclude that, under the conditions employed, 0.5 per cent of L-threonine is sufficient for the rat, but that 0.4 per cent is slightly inadequate.

Summary

The dietary requirement of the rat for L-threonine has been determined. The minimum amount necessary for maximum growth was found to be 0.5 per cent of the diet.

BIBLIOGRAPHY

1. McCoy, R. H., Meyer, C. E., and Rose, W. C., J. Biol. Chem., 112, 283 (1935).

2. Rose, W. C., Science, **86**, 298 (1937).
3. Rose, W. C., Bonner, P., and Rice, E. E., Unpublished experiments.
4. Rose, W. C., Oesterling, M. J., and Womack, M., J. Biol. Chem., **176**, 753 (1949).

VITA

The author was born of missionary parents on May 2, 1922, in Kulangsu, Amoy, China. He received his high school training at the Shanghai American School and at Williamson Central School, Williamson, New York. After receiving his diploma from the latter school in 1940, he attended Hope College in Holland, Michigan, and received the degree of Bachelor of Arts, with honors, from that institution in June, 1944. He was inducted into the Army of the United States in July, 1944, serving two years in the American Theater as a laboratory technician. In September, 1946, he began graduate work at the University of Illinois, where he held a half-time teaching assistantship for five semesters. He was granted a Master of Science degree in Chemistry by the University of Illinois in September, 1947, and held a University Fellowship in the academic year 1948-49. He held an Atomic Energy Commission Pre-doctoral Fellowship in Biological Sciences from July 1, 1949, to July 1, 1950. On May 31, 1947, he married Norma Winifred Lemmer of Kalamazoo, Michigan. He is the father of a son, Roger Erdman II, born July 1, 1949.

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